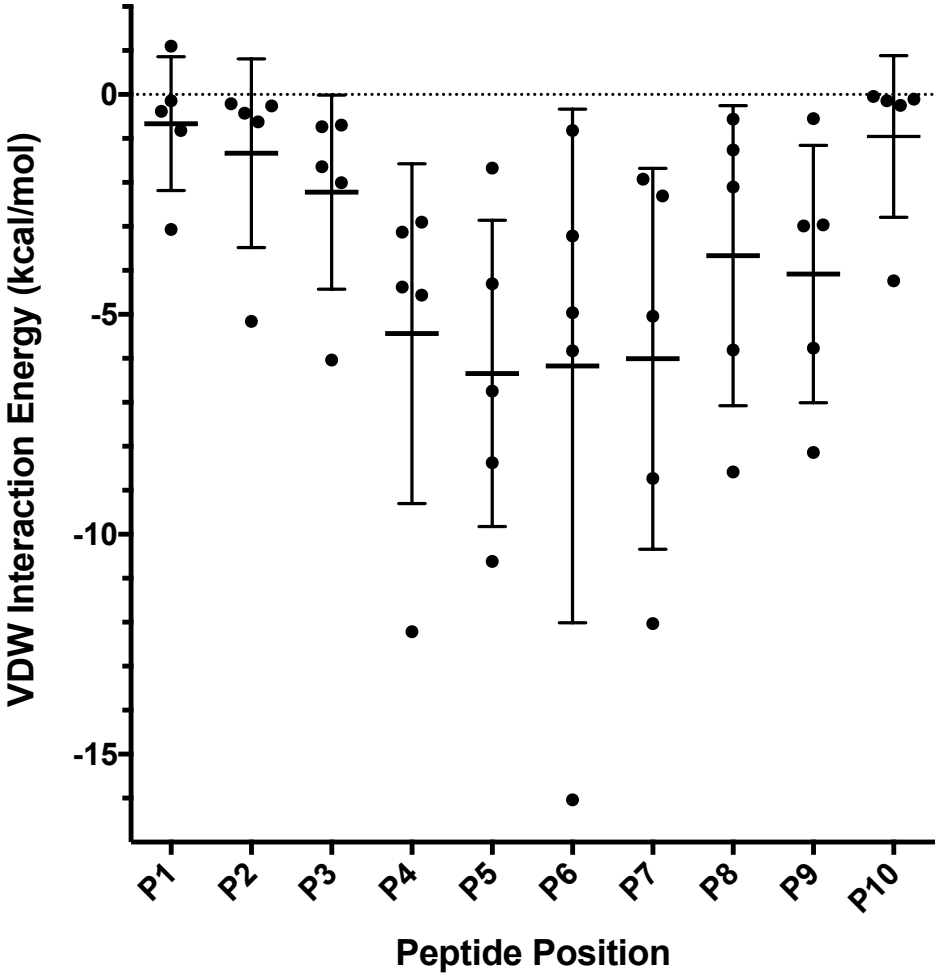
Supplemental Table 1. Unique co-crystal structures of human TCR:nonamer-pHLA class I complexes.

PDB	MHC	TCR	Peptide	Source
1OGA	HLA-A2	JM22	GILGFVFTL	Viral MP(58-66)
1AO7	HLA-A2	A6	LLFGYPVYV	TAX Viral Peptide
1BD2	HLA-A2	B7	LLFGYPVYV	TAX Viral Peptide
2BNQ	HLA-A2	1G4	SLLMWITQV	NY-ESO-1
3H9S	HLA-A2	A6	MLWGYLQYV	Tel1p
3PWP	HLA-A2	A6	LGYGFVNYI	Hud
3GSN	HLA-A2	RA14	NLVPMVATV	CMV-pp65
3QEQ	HLA-A2	DMF4	AAGIGILTV	MART-1
3QDJ	HLA-A2	DMF5	AAGIGILTV	MART-1
1MI5	HLA-B8	LC13	FLRGRAYGL	EBV/EBNA-3
3SJV	HLA-B8	RL42	FLRGRAYGL	EBV/EBNA-3
4QRP	HLA-B8	DD31	HSKKKCDEL	HCV/NS3-4A
3FFC	HLA-B8	CF34	FLRGRAYGL	EBV/EBNA-3
3KPS	HLA-B44	LC13	EEYLQAFTY	ABCD3

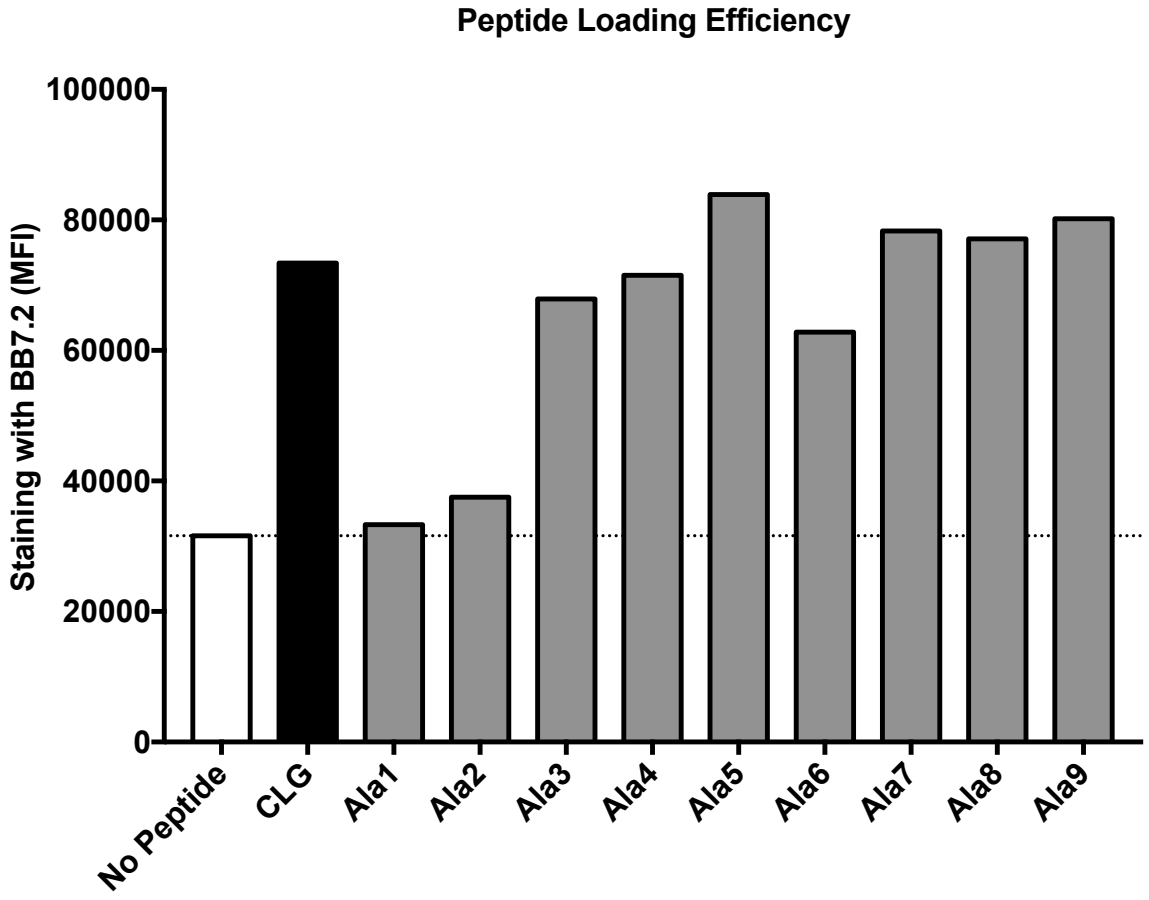
Supplemental Table 2. Unique co-crystal structures of human TCR:decamer-pHLA class I complexes.

PDB	MHC	TCR	Peptide	Source
3QDM	HLA-A2	DMF4	ELAGIGILTV	MART-1 (A2L)
3QDG	HLA-A2	DMF5	ELAGIGILTV	MART-1 (A2L)
3DXA	HLA-B44	DM1	EENLLDFVRF	EBV
3UTT	HLA-A2	1E6	ALWGPDPAAA	Insulin
3VXR	HLA-A24	H27-14	RYPLTFGWCF	HIV-1 Nef

Supplemental Figure 1. **Plot of calculated VDW contact energies of known unique TCR/decamer pHLA class I co-crystal structures showing a bell shaped distribution of contacts along peptide interface.** The peptide position (P1, P2, etc.) is indicated on the x-axis of the graph. Individual interaction energies from 5 different structures are plotted, with bars indicating mean +/- SD.



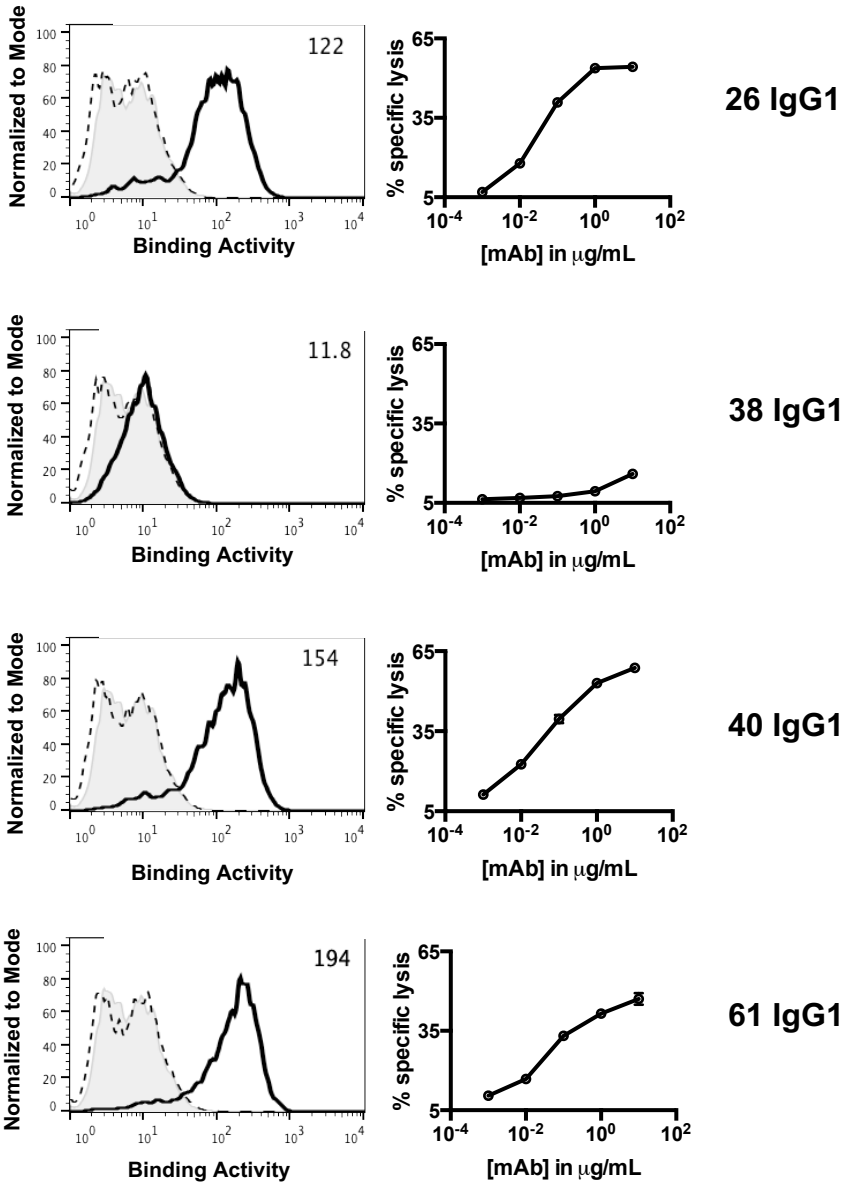
Supplemental Figure 2. **Peptide loading efficiency of Ala-substituted CLG peptides onto HLA-A*02:01.** T2 cells were pulsed with wild type CLG peptide or CLG peptides with Ala-substitutions at peptide positions P1 through P9, and then stained with BB7.2 which preferentially binds to HLA-A2 that is sufficiently loaded with peptide. CLG peptides with Ala at positions P1 and P2 do not load onto T2 cells.



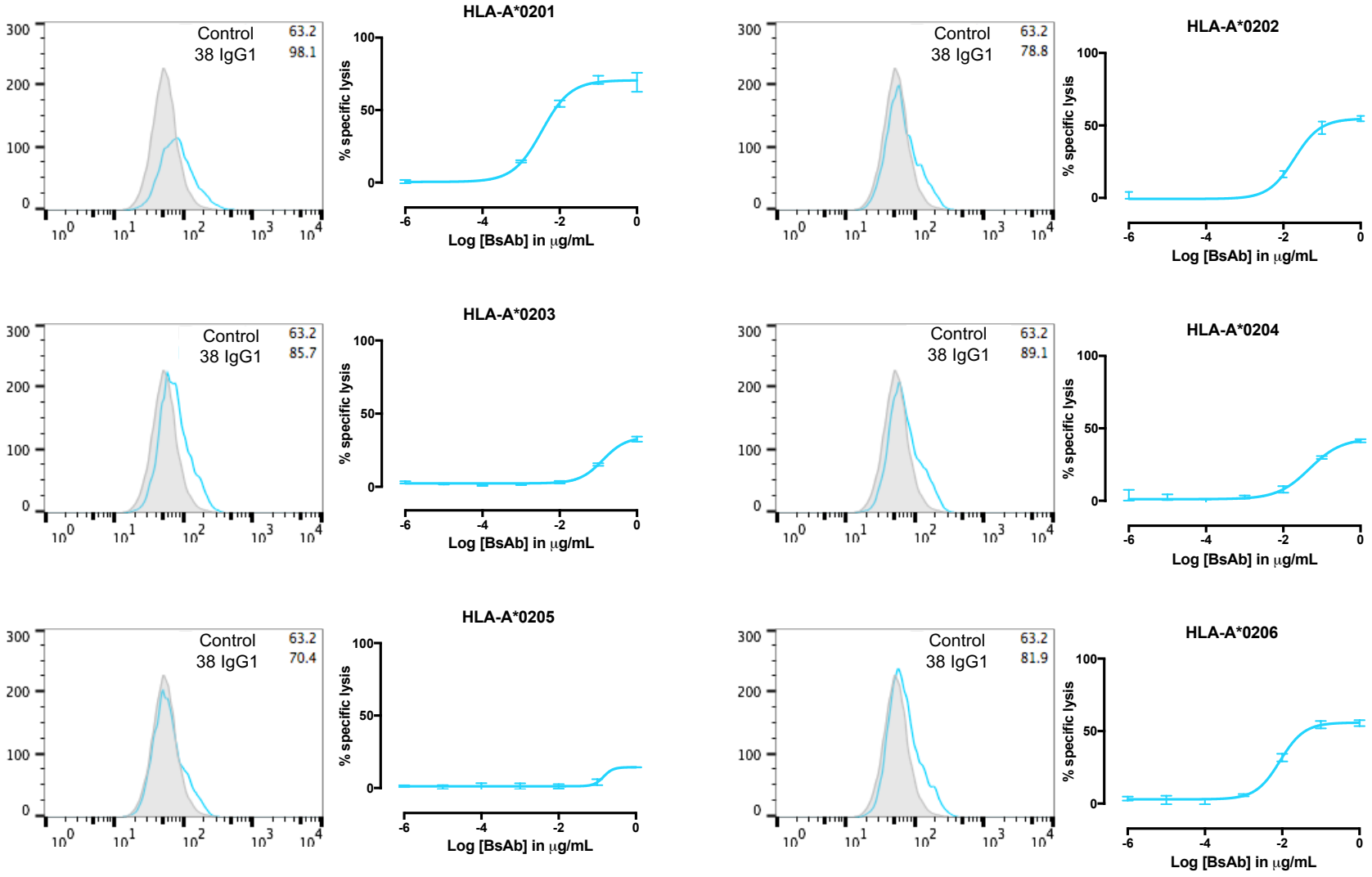
Supplemental Table 3. Binding kinetics of top 4 clones by surface plasmon resonance

Construct	$k_{on}(S^{-1}M^{-1})$	$k_{off}(S^{-1})$	$K_D(nM)$
26 IgG1	1.0×10^{7}	2.1×10^{-2}	2.0
38 IgG1	1.1×10^{4}	6.0×10^{-4}	52.2
40 IgG1	2.8×10^{7}	1.5×10^{-0}	52.3
61 IgG1	6.9×10^{6}	1.8×10^{-1}	26.4

Supplemental Figure 3. Binding and ADCC activity of top clones. Clones formatted to human IgG1 mediate ADCC against peptide pulsed T2 cells. T2 cells were incubated overnight with 100μM of peptide and 35 μM of β2M, in IMDM serum free media. Binding was determined by FACS (left) and activity was determined by ADCC (right) for each IgG1. ADCC used CD16-transfected NK92 cells as effectors. Effectors were incubated with pulsed T2 cells for four hours (20:1 E:T). Black line denotes CLG pulsed T2 cells, dotted black line denotes YML (irrelevant) pulsed T2 cells. Solid grey denotes an isotype control antibody.



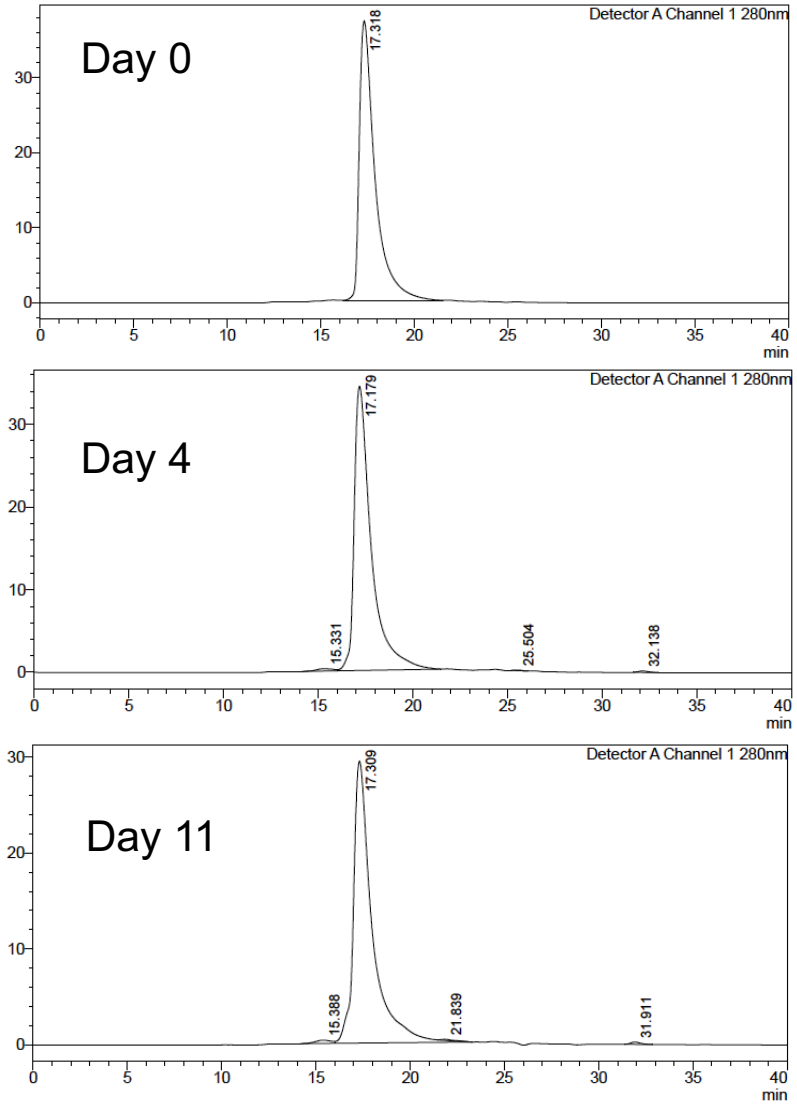
Supplemental Figure 4. HLA-A*02 suballele activity. Binding measured by flow cytometry using 38 IgG1 (left) and TDCC using tandem-scFv bispecific antibody (right) against peptide pulsed aAPCs expressing different suballeles of HLA-A*02 (01 through 06). Blue line denotes 38 IgG1 activity, grey plot is an isotype control. Data are from 3 technical replicates per experimental condition with mean ± SD plotted.



Supplemental Table 4. Biochemical properties of affinity matured versions of clone 38

Affinity matured clones	Retained specificity	ELISA enhancement	fold improvement		
			k_{on}	k_{off}	K_A
CLONE 38-1	yes	0.79	1.12	1.66	1.86
CLONE 38-2	yes	1.67	2.36	2.07	4.90
CLONE 38-3	yes	1.58	3.44	1.79	6.18
CLONE 38-5	yes	1.37	2.44	1.36	3.31
CLONE 38-6	yes	1.29	1.05	1.53	1.62
CLONE 38-11	no	2.65	6.15	1.38	8.46
CLONE 38-13	nd	0.55	2.35	1.23	2.89
CLONE 38-21	yes	0.74	1.44	1.68	2.42
CLONE 38 Parental	-	1	1	1	1

Supplemental Figure 5. **Stability profile of 38 DiBsAb**.
Clone 38 DiBsAb was incubated at 37°C for multiple days. Stability was determined by SEC-HPLC.



Supplemental Table 5*. In vitro* cytotoxicity of clones 38 and 38-2 DiBsAbs against HLA-A*02:01(+) EBV(+) targets. "nd" indicates "not determined" by non-linear regression analysis due to poor curve fit.

		38 DiBsAb	38-2 DiBsAb	Control
RPMI-6666	% Max Killing	20.7	46.8	11.9
	EC50 (µg/mL)	1.2E-02	1.9E-03	nd
DT BLCL	% Max Killing	39.9	55.1	9.2
	EC50 (µg/mL)	4.9E-01	2.2E-02	nd
F BLCL	% Max Killing	25.8	48.7	3.5
	EC50 (µg/mL)	3.3E-02	7.2E-03	nd
HONE-1-A2	% Max Killing	16.8	36.5	nd
	EC50 (µg/mL)	2.4E-02	7.4E-02	nd

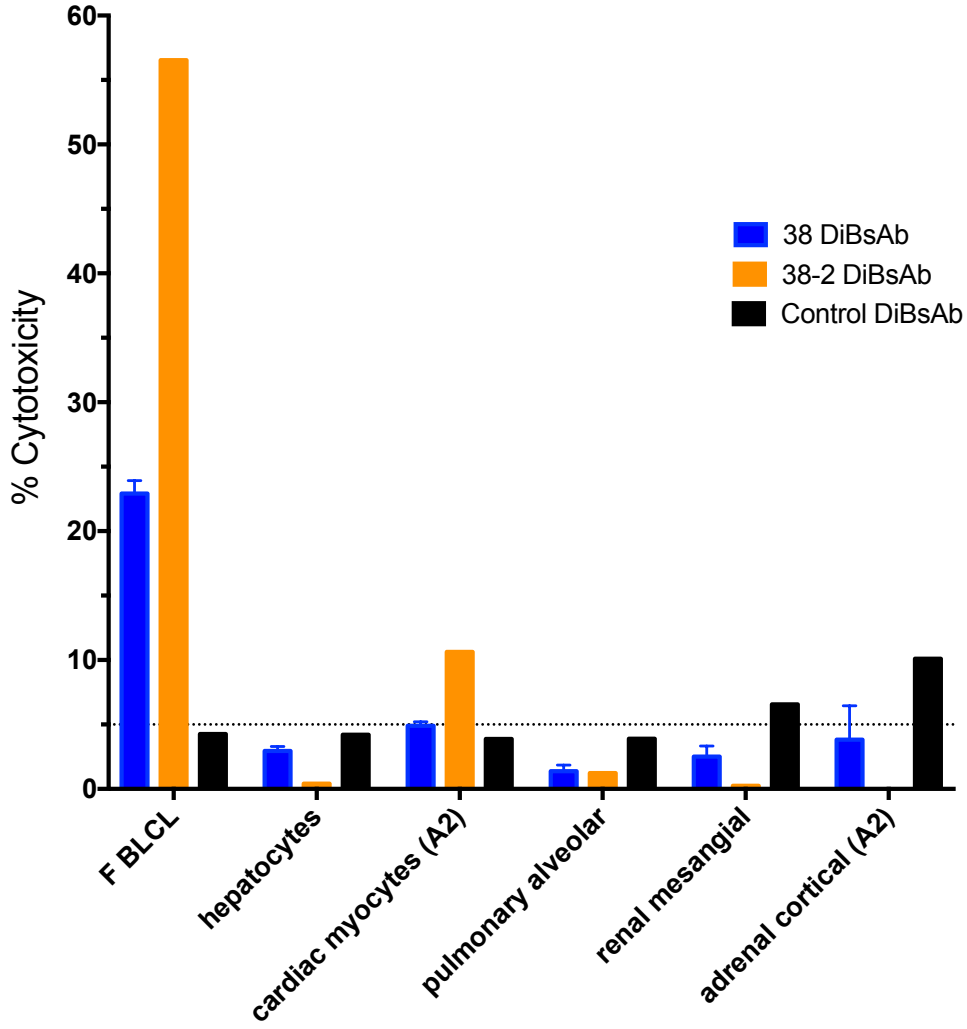
Supplemental Table 6. Analysis of tumor growth of F BLCL-luc in DKO mice treated with human adult PBMC and DiBsAbs

Groups	Luciferase Activity (AUC) d0-d28	% change, compared to Group 2	Fold change, compared to Group 2	p value, compared to Group 2
1. Tumor + Adult PBMC	$1.1\pm0.9\times10^{10}$			
2. Tumor + Adult PBMC + Control DiBsAb	$2.2\pm1.2\times10^{10}$			
3. Tumor + Adult PBMC + 38 DiBsAb	$2.6\pm0.5\times10^{9}$	-88.5%	8.8-fold	0.005
4. Tumor + Adult PBMC + 38-2 DiBsAb	$5.9\pm1.7\times10^{8}$	-97.4%	38-fold	0.003

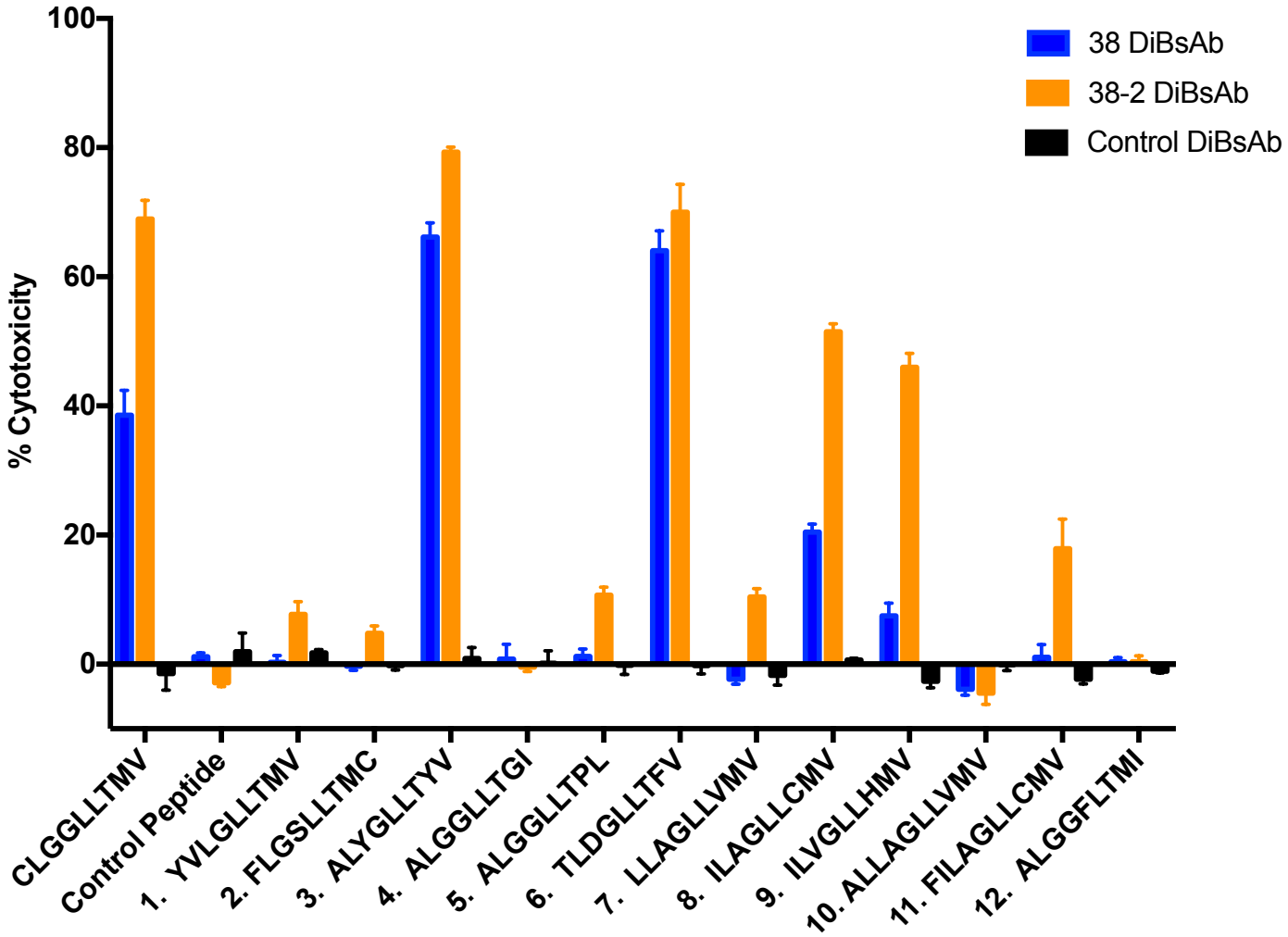
Supplemental Table 7. Analysis of tumor growth of F BLCL-luc in DKO mice treated with human cord blood PBMC and DiBsAbs

Groups	Luciferase Activity (AUC) d0-d28	% change, compared to Group 2	Fold change, compared to Group 2	p value, compared to Group 2
1. Tumor + cPBMC	$2.3\pm1.0\times10^{10}$			
2. Tumor + cPBMC + Control DiBsAb	$1.8\pm0.5\times10^{10}$			
3. Tumor + cPBMC + 38 DiBsAb	$4.2\pm2.0\times10^{9}$	-76.7%	4.3-fold	<0.001
4. Tumor + cPBMC + 38-2 DiBsAb	$2.6\pm 0.9\times10^{9}$	-85.3%	6.8-fold	<0.001

Supplemental Figure 6. Safety assessment of 38 and 38-2 DiBsAbs. DiBsAb activity was measured by TDCC against a panel of normal human cells. Each cell type was incubated with activated cord blood T cells (10:1 E:T) for four hours. 0.1 µg/mL of each DiBsAb was used. Blue denotes 38 DiBsAb, orange denotes 38-2 DiBsAb and black denotes the control DiBsAb. Data are from 3 technical replicates per experimental condition with mean ± SD plotted.



Supplemental Figure 7. Peptide cross-reactivity screen. Proteomic analysis identified 12 potentially cross-reactivity CLG-like human peptides (X-axis) that show strong sequence similarity to CLG peptide and are predicated *in silico* to bind to HLA-A*02:01. TDCC with 0.01 µg/mL of 38 (blue), 38-2 (orange) or a control (black) DiBsAb were done on the respective peptide-pulsed T2 cells using activated T cells derived from cPBMC (10:1 E:T ratio). T2 cells were pulsed with 10µM peptide and 3µM β2M for 1hr at 37°C in IMDM serum free medium. Cytotoxicity was measured after 4hrs. Data are from 3 technical replicates per experimental condition with mean ± SD plotted.



Supplemental Table 8. Selection of CLG-like peptides for potential cross-reactivity analysis. Peptides shown in red were found to be cross-reactive to 38 DiBsAb. The top 12 peptides were identified based on their similarity to the CLG peptide (columns 4 and 5) and their predicted binding to HLA-A*02:01 (column 3, lower IEDB score reflects better binding). Peptides in grey were not included due to their low likelihood of binding HLA.

Peptide No.	Peptide	IEDB	Total No. of matching residues	No. of matching residues in GLLTM epitope	Gene symbol
WT	**CLGGLLTMV**	1.3			LMP2a
1	**YVLGLLTMV**	0.5	6	5	MOSPD3
	NSGGLLTMS	57	6	5	STK31
2	**LLAGLLVMV**	0.5	6	4	GPR78
3	**ALYGLLTYV**	0.3	6	4	ARMC5
4	**ALGGFLTMI**	2	6	4	GPR34
5	**ALGGLLTGI**	2.4	6	4	SLC5A12
6	**ALGGLLTPL**	1	6	4	TMEM161A
7	**ILAGLLCMV**	0.5	6	4	CLDN14
8	**TLDGLLTFV**	0.4	6	4	LILRB5
9	**ILVGLLHMV**	0.4	6	4	ORMDL2
10	**ALLAGLLVMV**	0.25	6	4	GPR78
11	**FILAGLLCMV**	0.25	6	4	CLDN14
	GLGGLLTDK	37	6	4	AP5B1
12	**FLGSLLTMC**	3.6	6	4	HERC2
	VLGGLLKMC	19	6	4	SLC9C2
	PDGGLLPMV	35	6	4	hCG1818423

Supplemental Table 9. Cross-reactive peptides for Clone 38 DiBsAb and their respective gene product precursors.

Peptide No.	Peptide	Gene Symbol	Gene Description	Expression
3	**ALYGLLTYV**	ARMC5	Armadillo repeat containing 5	Present on U2OS and MCF7
7	**ILAGLLCMV**	CLDN14	Claudin 14	Present on Liver cells
8	**TLDGLLTFV**	LILRB5	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 5	Present on PBMC
9	**ILVGLLHMV**	ORMDL2	ORMDL sphingolipid biosynthesis regulator 2	Present on U2OS and MCF7